Biology 337 *NAME:* ________________________ **Lab worksheet**
Biology of Invertebrates *LAB TIME:* ____________________ **Dr. Bob Podolsky**

LAB D. Ph. NEMERTEA, Ph. NEMATODA, & Ph. ANNELIDA (Cl. Polychaeta)

I. Ph. NEMERTEA ("never-erring" or Ph. Rhyncoceola, "snout cavity"; ribbon worms)

Nemerteans (or "nemertines") are active, benthic predators that use an **eversible** sticky, entangling, or barbed/poisonous **proboscis** to capture prey. The proboscis is inverted by a retractor muscle into a fluid-filled body cavity called the **rhynchocoel**. The proboscis and its rhynchocoel are a shared, derived character for the phylum. To use the proboscis in prey capture, the rhyncocoel is squeezed through the contraction of circumferential (circular) muscles, forcing the proboscis to evert forcibly under high hydrostatic pressure.

A. Taxonomy. Traditional taxonomy divides the phylum into two classes:
Cl. Enopla: possess a hard, piercing **stylet** at the tip of the proboscis; proboscis pore and mouth are joined as one opening; mouth opens anterior to the brain
Cl. Anopla: lacks a stylet on the proboscis; proboscis pore and mouth are separate; mouth opens posterior to the brain

TQ: Some more recent phylogenies place the Enopla <u>nested within</u> the Anopla. Draw two simple phylogenies for the taxa, one showing the traditional arrangement and one showing the more recent one. What does the latter imply about the –phyly of each class and whether the absence of a stylet is ancestral or derived?

B. Exercises

1. Examine the two w.m. slides of a small nemertean. (No extra slides are available.)

- **Slide A** will be focused on the anterior end of the inverted proboscis. The tissue will change to a thinner width and lighter pink color where the tip of the proboscis connects to the long **retractor muscle** that withdraws the tip into the rhynchocoel. Be sure you understand how proboscis *withdrawl*, *inversion*, and *eversion* work, including which muscles are involved.
- **Slide B** will be focused at higher power on the inverted proboscis tip. By focusing up and down you will see the functional **stylet** associated with its **poison gland**, as well as two groups of reserve stylets sitting to the side within **styletocytes,** the cells that form new stylets. To which class does this specimen belong? ___________

2. Examine the preserved specimen(s) of *Cerebratulus lacteus*.

- The larger specimen shows the great length these animals can achieve, far beyond flatworms, nematodes, or most polychaetes. The body is also flattened like a "ribbon." As in flatworms, the epidermis has no cuticle, is ciliated and contains secretory gland cells that produce mucus. The body is featureless, other than some head features (see below) and a terminal anus.
- The smaller specimens show three distinct head features. (1) The **mouth** is a large ventral slit or cleft. (2) The pair of **cephalic slits** on either side of the head open into small chambers. Cilia circulate water past **chemosensory organs** within the slits. (3) On one specimen, the **proboscis** is extended; on the other, locate the **proboscis pore**, a midventral opening anterior to the much larger **mouth**. To which class does this specimen belong? ___________

3. <u>If</u> live animals are available, note several distinct eyespots in the head region (difficult to see in preserved specimens). Under the right light for some specimens you might be able to visualize the rhyncocoel and proboscis through the body wall. Deduce the mechanism of movement—do they glide as if using cilia, or undergo shape change as if using muscles, or a combination?

TQ: Nemerteans are the first phylum you've examined in lab with a so-called "complete" digestive system (possessing mouth and anus). Why might a complete digestive system be more effective or efficient than an incomplete digestive system? Give three specific reasons.

II. Ph. NEMATODA ("thread body")

The 20-100,000 known nematode species are found in nearly every moist environment--terrestrial, marine, and freshwater. They are the most abundant multicellular animals on the planet, with as many as 4 million per square meter (!) in some marine habitats. About half of all species are free-living, moving among sediments and feeding on bacteria, fungi, and other microorganisms. The other half includes diverse plant and animal parasites, many of which are medically or agriculturally important. Most nematodes are long, slender, almost featureless, externally tapered at both ends, and *very round* in cross section (why *so* round?).

The body cavity is a "pseudocoelom" derived from the embryonic **blastocoel**—it is not lined by an epithelium and functions as a **hemocoel** (blood cavity) that bathes the internal organs. The **high internal pressure** of the body cavity is resisted by a **chitonous cuticle** reinforced by **collagen fibers**, which aids the animal in its characteristic **sinusoidal** locomotion. Contraction of well-developed **longitudinal muscles** flex the body in a sine wave; circular muscles are **<u>absent</u>**. The cuticle, secreted by the **syncytial** epidermis, must be **molted** during development.

The nervous system includes a prominent ring around the esophagus with several **longitudinal nerve cords**. In an unusual design feature, rather than nerves sending motor neurons to muscles, the muscles instead send long cytoplasmic processes to the nerve cords. Nephridia are absent, and the elimination of metabolic wastes (ammonia) is mostly by **diffusion** across the body wall. Osmoregulation, however, is sometimes accomplished by an **excretory gland and canal system**, particularly in species that live in freshwater environments (why?).

TQ: As just explained, most excretory systems solve two general physiological problems. (1) Name them. Then explain (2) why one of these problems <u>must</u> be solved by an excretory system even though (3) the other problem can sometimes be solved without it. (*Hint: consider how concentration gradients are involved in diffusion)*

A. HIGHER TAXONOMY

The phylum Nematoda is a member of a larger clade (a "superphylum") of phyla, most of which share a set of derived features (and which were formerly lumped with a set of unrelated species into the "pseudocoelomates"). These features include (1) a **pseudocoelom**, (2) a thick, extracellular **cuticle** that is **molted** during development, (3) the **loss of locomotory cilia** (even in sperm, which move like amoebae!), (4) the **loss of circular muscles**, (5) a fixed number of cells in the body (a phenomenon known as '**eutely**,') and (6) a **ring-shaped brain** that encircles the anterior foregut—hence, the superphylum name **Cycloneuralia**. Locate this superphylum on your phylogenetic roadmap, and note its position within the Ecdysozoa (molting animals).

If live nematodes are available, examine them in a dissecting microscope to note the sinusoidal motion characteristic of the phylum. Recall that nematodes lack circular muscles, so body movement must result from the alternating contraction of longitudinal muscles in any given section: first **dorsal muscle bands,** then **ventral muscle bands**, and so on. Nematodes use this motion to move waves down the body, pushing against sediment or fluid.

TQ: Given the alternating use of muscles as described, does the nematode create propulsive waves side-to-side (like a shark) or up and down (like a dolphin or whale)?

III. Ph. ANNELIDA ("ringed")

Like many phyla, annelids are worm-shaped, but in addition possess an extensive fluid-filled **coelom**. In many groups the body and internal coelom are built from a series of segments, which give the body its "ringed" appearance (hence the phylum name). Body segments also have a series of repeating external features (like **parapodia**) and internal features (like blood vessels and metanephridia). This serial repetition of many body sections is known as **metameric segmentation**. Segments are divided internally by **septa** (thin walls of connective tissue and peritoneum), which allow sections to function somewhat independently in locomotion, reproduction, and excretion. In addition, the coelom in each segment is often divided longitudinally by a **mesentery** (like a septum but oriented parallel to the anterior-posterior body axis).

A. TAXONOMY

Taxonomy is under heavy revision. Traditionally the phylum included two classes, the largely marine Cl. Polychaeta and the largely freshwater/terrestrial Cl. Clitellata (made up of two major subclasses, Oligochaetes and Hirudinea). More recently, the phyla Echiura and Sipuncula have been recognized potentially as additional classes within the Annelida.

Cl. Polychaeta. Primarily marine, 70% of all annelids. The parapodia are often associated with many setae (or chaetae—hence the name). Lifestyles are diverse but can be classified, to start, as errant (highly mobile) or sedentary (often tube-dwelling). Most species have separate sexes and a planktonic, trochophore larva (e.g. *Nereis*).

Note: The most interesting taxonomic level for diversity among the polychaetes is the **family**. In this lab you will examine many families and the relationship between their morphological and ecological traits. I do not require that you learn all the family names (as would be the case in a traditional course) but as a self-respecting invertebrate biologist you ought to, and you might garner extra credit for knowing those that we emphasize. One special family is mentioned below because until recently it was recognized, like the echiurans and sipunculans (see below), as a distinct phylum.

F. Siboglinidae. The vestimentiferans and pogonophorans, poorly known until the 1970s when deep-sea research developed significantly, live at cold methane seeps or hydrothermal vents. These animals gain nutrition through chemosynthesis by symbiotic bacteria housed within a specialized structure, the **trophosome**. The trophosome lies in place of a digestive system, and these animals otherwise do not feed. The possession of setae and the partitioning of the coelom only in the very posterior-most body region (often missed by early collection methods) indicate their affinity with annelids.

⇩*Note: You are responsible for the taxonomy of the following taxa only to the extent they are covered in lecture.*

Cl. Clitellata. The clitellum—a specialized multi-segment body region that is a shared derived character for this class—secretes a cocoon in which reproduction and/or development takes place. All are hermaphroditic.

Subcl. Oligochaeta. Lack parapodia, have few setae, segmented. Can be freshwater or marine, but most familiar members are terrestrial (e.g., earthworm *Lumbricus terrestris*). Most locomote using peristaltic waves of contraction along the length of the body.

Subcl. Hirudinea. The leeches lack parapodia, have no setae, and can appear segmented externally although the coelom is not subdivided internally. Most are ectoparasites. Most live in freshwater, though some are terrestrial. They have two suckers and locomote via looping (e.g., the medicinal leech *Hirudo medicinalis*).

Cl.(?) Echiura. Members lack parapodia, are not segmented as adults (but show traces during development), and possess only a pair of setae on the body surface. They live in sandy or muddy burrows and use a non-retractable proboscis for deposit feeding (e.g. the sausage worm *Echiurus echiurus*). A more unusual example is the fat innkeeper worm *Urechis caupo*, which excavates burrows occupied by many commensals (hence the common name), and produce a mucus net that traps particles as the animal ventilates the burrow using peristaltic contractions of the body. *Urechis* is also unusual for a pair of anal sacs that are used for gas exchange and excretion.

Cl.(??) Sipuncula. Members lack parapodia, chaetae, and any trace of segmentation. Their most notable feature is a fully retractable, eversible **introvert** at the anterior end of the body with a mouth and tentacles powered hydraulically by their own coelomic **compensation sacs**. Sipunculans may ultimately wind up being classified as polchaetes, a class related to polychaetes, or a separate phylum.

B. Family-level diagnostics

In lab we will focus on the largest and most common marine class, the **polychaetes**, which show enormous diversity. In polychaetes, unlike in most other groups we will study, this diversity is most clearly distinguished at the *family* level. That is, families show characteristic features that distinguish them from other families. There exist a very large number of polychaete families, only some of which you will observe here. The key to getting a handle on this diversity is to study how habitat and feeding mode can be deduced from three morphological features:

(1) *head shape and appendages*: species that move on or near surfaces typically have prominent **sensory structures**, whereas burrowers often have less conspicuous sensory structures. Animals that live burrowed, often in anoxic sediments, may have prominent **gills** (outgrowths of the body wall), especially concentrated near the anterior end. Finally, **trophic structures**, such as jaws, often bear a direct relationship to feeding mode and habitat.

(2) *parapodium size and shape*: large parapodia with stiff chaetae are typical of errant polychaetes that move on surfaces; paddle-shaped parapodia may be found in species that swim; and smaller parapodia are typical of those that burrow or live in tubes (peristaltic body movements, not parapodia, are the main mechanism for burrowing).

(3) *segment differentiation*: segments are typically similar along the length of a polychaete body, but some groups show a tendency toward **tagmatization**, or the regional differentiation of segments to form distinct body parts. For example, the chaetopterids are subsurface suspension feeders that use specialized parapodia to collect and transport food particles.

Your overall goal is to understand how a basic body plan has been modified in small ways for different habitats and lifestyles. Simple drawings and notes will help you to organize your understanding of how these major features relate to lifestyle differences.

C. EXERCISES

You will examine the features described above in preserved and live specimens from several polychaete families. To help in making these family-level identifications, you should consult the supplementary handout, "Lifestyles of the Wet and Slimy," as well as A Polychaete Primer, created by students in a similar course in a far-away place long ago. For additional help, see this interactive online site for family identification: http://rmbr.nus.edu.sg/polychaete/frame.html

MOBILITY

In the following exercises, in addition to studying the feeding guilds of different polychaete families, start by dividing the polychaetes roughly into two groups: the "errant" polychaetes (= active, either crawling or burrowing) and the "sedentary" polychaetes (= live in tubes or crevices). Here are some key features to watch for among the specimens available:

"Errant" polychaetes: Typically active with large parapodia, abundant sensory structures on the head, and little regional specialization of body segments. As the name implies, they seek out food rather than collect it locally, so tend to include scavengers and predators rather than suspension or deposit feeders.

"Sedentary" polychaetes. Typically live in tubes, crevices, or virtually permanent burrows. Commonly with fewer head structures, reduced parapodia, and sometimes specialization of body segments. Many possess long feeding tentacles that extend from the mouth of the tube or burrow to collect particulate food, either as detritus feeders, deposit feeders, or suspension feeders, although some are sit-and-wait predators. See examples of tubes later in the lab.

TQ: What are two a major challenges of living inside a confining tube or crevice? What additional features or behaviors of sedentary worms can help to avoid these problems?

FEEDING GUILDS

These exercises focus on one example of each of six feeding guilds. See the "Lifestyles" guide for more information.

1) Herbivore/omnivore/scavengers: F. Nereidae

Nereis is an **errant** herbivore with prominent head and parapodial features. It is traditionally emphasized because of these features and because live specimens can usually be collected.

Preserved specimens and prepared slides

- Using your dissecting scope, examine two specimens of *Nereis* in a dissecting tray covered with water. Choose one with its **pharynx** everted, and the other with its pharynx withdrawn. Identify the **prostomium** (small, non-segmental region in front of the mouth), **peristomium** (segment on which the mouth is located), **eyes** (dark, glassy structures), slender **sensory tentacles**, and thicker **sensory palps**.
- Examine the head region on the w.m. slide of *Nereis*. In this specimen the pharynx is not everted, but you can see the jaws and pharynx through the body wall to get an idea of its position when withdrawn. (note: only 1 slide available)
- Examine the w.m. slide of a *Nereid* parapodium. Identify the **dorsal cirrus**, **notopodium**, **neuropodium**, **chaetae**, and **aciculae**. (note: only 1 slide available)
- Examine a prepared slide of a *Nereis* cross section. Draw and identify the following structures: thin **cuticle**, **epidermis**, stringy **circular muscle layer**, thick bundles of **longitudinal muscle**, spacious **coelom**, **dorsal** and **ventral blood vessels** (may appear as red spots), any remnants of **mesenteries** that divided the coelom and held the gut in place, **gastrodermis** lining the gut, ventral paired **nerve cords**, and **muscles** in the lateral **parapodia**. In some sections you will also see sectioned pieces of **acicula**, the thick chitinous supporting fibers within the **parapodia**.

Living specimens

Locomotion. Carefully watch locomotion of *Nereis* in a dish, using a probe to orient it for viewing. Examine the prominent head sensory structures: slender **tentacles**, **eyes**, and thick **palps** "tasting" the substrate.

TQ: Examine how the large parapodia are coordinated during movement. What is the direction of the metachronal wave? If you observed ctenophores earlier, how does the direction of the metachronal wave of parapodia and ctenes compare? State a hypotheses for why both types of structures have metachronal waves that move in the directions observed.

Blood flow. Carefully observe and sketch the pattern of blood flow in the dorsal major vessel and, if visible, in other vessels that arise from this major vessel, especially near the head.

TQ: Based on blood flow you can see in the dorsal vessel, deduce which direction blood must flow in the ventral vessel.

TQ: Now, recall from your notes the direction of flow within each segment. Based on this pattern of circulation, which major vessel (dorsal or ventral) do you think carries (1) more oxygen? (2) more nutrients? State a hypothesis for why blood travels in these particular directions in the two major blood vessels.

2) *Predators*: F. Glyceridae

Glycera is an **errant, burrowing** carnivore with reduced head and parapodial structures that relate to its burrowing lifestyle. These animals are fierce predators with an eversible **pharynx** and venomous **fangs**. They burrow through sediment, creating a series of interconnected tunnels, in search of infaunal prey, using the powerful pharynx to penetrate the sediment and the prostomium as an anchor. The jaws are exposed in the preserved specimen.

Two other remarkable, closely related families of errant predators are worth seeing:

F. Polynoidae. The "scale worms" are characterized by soft scales, called **elytra**, on the dorsal surface. These scales are easily autotomized (what does this term mean?) when disturbed by a predator. As in *Nereis*, note the large parapodia, lack of body regionalization, and prominent sensory structures on the head. Members of this family are sometimes commensals, nestling within the external cavities of molluscs or echinoderms and running out to subdue prey.

F. Aphroditidae. These large, slow moving worms, known as "sea mice," live on soft muddy bottoms where they plow through sediment and consume small animals. The body is covered with **elytra** that are hidden behind a dense mat of poisonous, irridescent setae, which can be erected when in danger like a porcupine or echidna.

3) *Suface deposit feeders*: F. Terebellidae.

Amphitrite is a **sedentary, surface deposit feeder** that builds soft mud tubes on surfaces or in sediment, and sends out long spaghetti-like tentacles across the surface to transport sediment particles back along ciliated grooves on the tentacles.

> *Live specimens*. Examine the gills, which will be bright red and highly active to increase water flow. The gills contain hemoglobin, showing extensions of the **blood-vascular system**. Look also for circulation in the lumen of the tentacles, which have extensions of the **coelom**.
>
> **As with all live specimens of tentacled feeders,** you can try to squirt a little carmine particle suspension on the tentacles and watch them capture and transport particles down the ciliated food groove to the mouth.

TQ: To answer this question, examine the two graphs given in the section on terebellid worms in your handout "Lifestyles of the Wet and Slimy." First digest the meaning of the x-axis of each graph: a selectivity index, which is the degree to which a given particle size was selected for tube building or for ingestion *relative to* its abundance in the sediment. Positive values mean that a particle size was selected in greater proportion than its abundance, and negative values mean that it was selected in lower proportion than its abundance.
(1) What do the graphs indicate about selectivity of particles for building tubes and for feeding?

(2) Propose a functional reason why the particular particle sizes were chosen for feeding. (*Hint: your answer should invoke a scaling argument about the size of particles ingested: what nutrition does the animal actually get, and what does it have to ingest to get it?)*

4) ***Sub-suface deposit feeders*****:** **F. Arenicolidae**.

Aberinicola "lugworms" are common sedentary **burrowers** in mud/sand flats. "Fecal castings" are commonly depositied at the surface of their burrows. Find the very reduced parapodia and note the lack of sensory structures on the head. The prominent gills (derived from modified parapodial cirri) are located in specific regions along the body. Do they appear to be derivatives of the **notopodium or neuropodium**? **(circle one)**

5) Muco-ciliary suspension feeders**:** **F. Sabellidae**

"Fan worms" possess of a crown of long, pinnate (featherlike) feeding tentacles called **radioles** at their anterior end, and have reduced parapodia. The tentacles are extended into open water where they collect food particles from suspension with mucus, and use an elaborate set of ciliated grooves to transport particles to the mouth. Photosensitive cells on the radioles and giant nerve fibers in the body enable the fast retreat of tentacles into the tube when a shadow passes.

Members of this fan worm family build soft, parchment-like tubes that are commonly seen protruding from sandy substrates. If a preserved specimen is available, note the groove running down the length of the body, which houses cilia that beat from the anus to the head, helping to carry fecal waste out of the tube.

6) Sub-surface suspension feeders**:** **F. Chaetopteridae**

Perhaps the most unusual polychaete family of all, the chaetopterids construct a U-shaped tube in the sediment and pump water through the burrow to capture suspended food particles. Unlike the fan worms, which capture food using mucus and cilia on bushy tentacles, these animals secrete a mucus bag that they suspend and use to trap particles. The bag is periodically consumed. A pair of specialized "arm" parapodia are used to hold the mucus bag, while other specialized "fan" parapodia act as paddles to pump the water through the burrow. As a result, the body shows the highest level of regional specialization ("tagmatization", which you will see prominently in the arthropods) among polychaetes.

TUBE CONSTRUCTION

As already noted, many sedentary polychaetes construct tubes as dwellings. Examples already mentioned include **F. Terribellidae** (**surface deposit feeders** in ***soft mud tubes***), **F. Sabellidae** (**suspension feeders** in ***soft parchment tubes***), and F. Chaetopteridae (**sub-surface suspension feeders** in ***soft parchment tubes***). The following four additional families will be used to illustrate different types of ***tube construction*** rather than features of the animal, although **feeding guilds** are mentioned.

F. Onuphidae. Unlike almost all other sedentary species, this family is **predatory**, lunging out of its tube when prey come near. As a consequence, they have some features that we associate with errant species, such as well-developed sensory structures, large parapodia, and large jaws. The upper portions of tubes of a local species, *Diopatra cuprea*, are extremely common on mudflats and muddy stretches of beach. The tubes are composed of a ***secreted, parchment-like material*** with mud and debris attached.

If live animals are available, observe the very large jaws used as part of their sit-and-way predatory strategy, and note the iridescence of the multilayered cuticle that refracts light like a prism.

F. Oweniidae: These **surface deposit feeders** include the local species shown that constructs tubes from a mixture of ***sand, mucus, and carefully chosen shells*** that overlap like shingles.

F. Pectinariidae. A local species, *Cistenides gouldii*, known as the "ice cream cone worm," is a **sub-surface deposit feeder** (see "Lifestyles" guide) that constructs elegant conical tubes from ***large, uniform sediment particles*** that are carefully aligned to give a smooth surface.

F. Serpulidae. These **suspension feeders** secrete ***hard*** $CaCO_3$ tubes, typically on other hard surfaces such as empty mollusc shells. The tubes form no predictable shape. If a live specimen is available, find the umbrella or plug-shaped **operculum** that is used to close off the tube opening from predators after the tentacles are withdrawn.